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MOLDS IN BAKERIES
Their Source, Varieties and Method of Prevention

by
J. W. Streider and R. N. McClellan

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Molds in Bakeries

Their Source, Varieties, and Method of Prevention

**A Report on Investigations Conducted for the American Institute of Baking
Under the Direction of**

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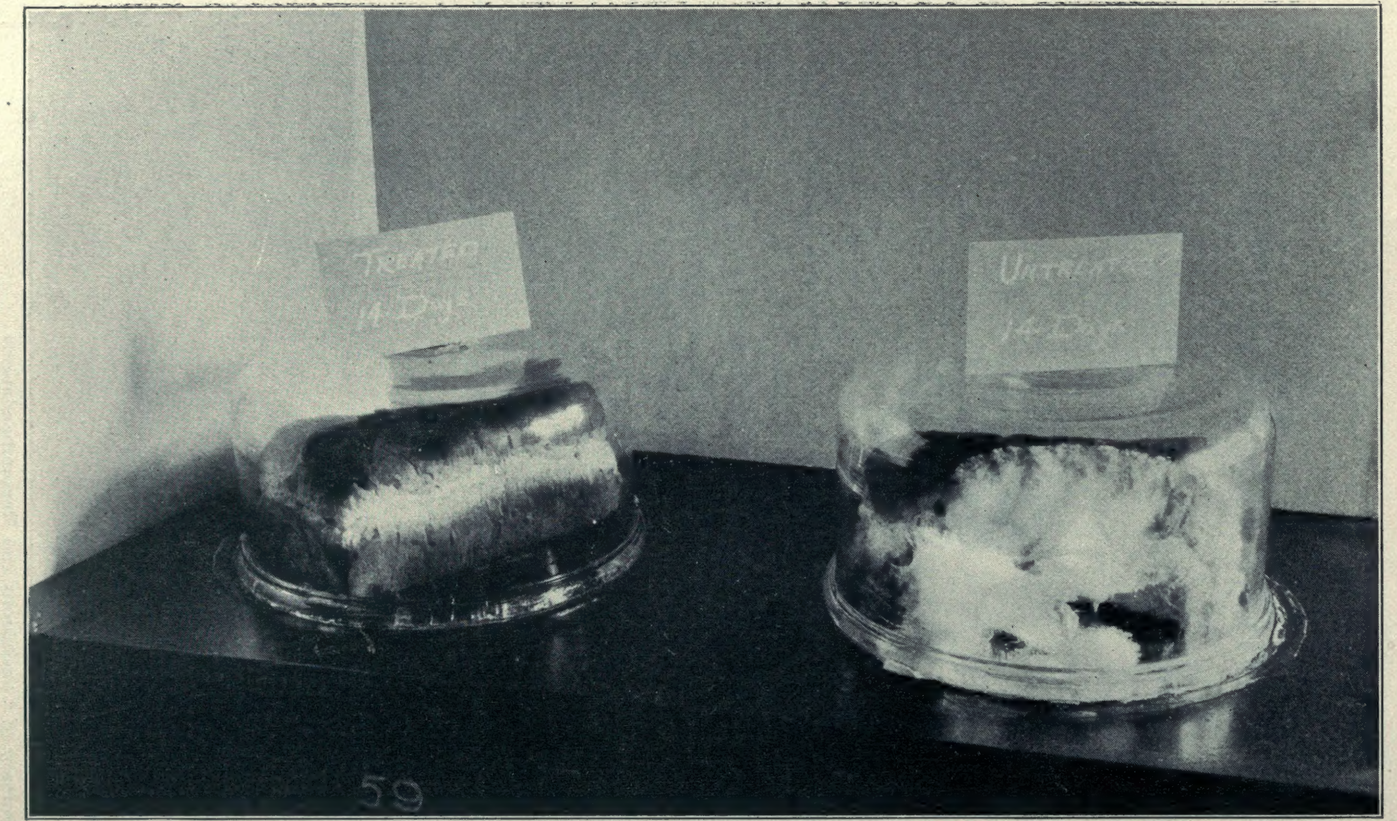


MOLD INFECTED BREAD

Showing the effect of heat treatment as a curative.

Molds in Bakeries

Introduction

ACCORDING to the latest figures of the United States Bureau of Census, there are, at the present time, 24,919 establishments in the United States engaged in the baking of bread. These establishments involve a capital of $417,017,457 and employ the services of 159,283 persons. At this time, the industry ranks seventh or eighth, having come up from twenty-second in 1905.

The enormous increase in the use of bakery-made bread as distinguished from that prepared in the home is one of the striking developments of the past few years. No other article of food is so universally employed as bread, and it is therefore evident that none deserves more adequate consideration as to the character of raw materials and protection during the processes of manufacture and handling. Great advances have already been made in these respects, with the result that the bread of today is undoubtedly far superior to the bakery product of a few years ago, while the protection of loaves by wrapping and by transportation in containers, which eliminate large quantities of dust and contact with unclean fingers, has gone far to assure the consumer of a clean and sanitary product.

One of the problems which has been of considerable importance in the study of bread-making methods pertains to the molding of bread. This seems to occur at unexpected times, and even under conditions which are supposed to eliminate largely or entirely the possibility of contamination from dirt and dusty air, and it has not been entirely clear to those who have studied the situation as to what the conditions are which determine the molding that has been observed.

The information which is generally accepted among those engaged in the industry may be briefly correlated. Bread molds very quickly in hot and humid districts, and infection is most prevalent during the summer months. Bakers on the Atlantic seaboard are nearly always in trouble during the summer and the trouble also occurs frequently in the central states during the months of June, July and August. Occasionally loaves will mold during the winter months when the bread is wrapped warm and handled under conditions which are presumably favorable for the molding. However, this does not always happen, hence there is a need of definite knowledge.

According to Jordan and other authorities, the eating of slightly moldy bread does not seem to be accompanied by any serious injury to health. At the same time the recorded instances of occasional illnesses attributed to this cause are sufficiently numerous to warrant caution in the use of bread in or on which molds of any kind are growing. Penicillium, Rhizopus, and Aspergillus are the fungi that most commonly attack bread, and there is some evidence indicating that poisonous or pathogenic races of these organisms may exist in some localities. Certain Italian observers claim to have extracted toxic substances from both the spores and the hyphae of some of these fungi, while German workers have eaten quantities of moldy bread without ill results.

Most bread reaches the retailer within twenty-four hours after it leaves the oven. In rare cases this time is extended to thirty-six hours. Some western bakers ship bread into a territory fifteen hundred miles across. This is particularly true during the harvesting months in the grain districts. As far as is known, the farthest shipments go about eight hundred miles. More bread is shipped in the summer than in the winter, but year by year the bread business is becoming more stabilized. The total amount of loss due to molding is, of course, but a fraction of the total output and yet in certain parts of the country the loss is really such a serious one that its study is distinctly important.

It would seem from the foregoing as if this molding of bread were a question of weather, humidity, and temperature, yet these alone are but favoring conditions and do not include the most essential factor, the causative agent itself, for obviously, no molding could take place without microbic infection from some source.

The problem we have undertaken, therefore, has the following salient points on which exact information is necessary in order to arrive at conclusions which may be applied in baking practice:

(1) The causes of molding, i. e., the particular organisms involved.

(2) The sources of the infection.

(3) The methods by which contamination of the bread takes place.

(4) Methods of prevention or inhibition of the infection.

To secure the facts on these and other important matters connected with this problem, we have made a systematic study of the baking industry.

Although the conditions existing in smaller bakeries were studied to some extent, it was felt that greater service to the industry as a whole could be secured by placing greater stress on the larger sources of supply, and a more exhaustive study was therefore made of some of the modern plants using large scientific methods of control of operations. It is in institutions of this type that the large losses are sustained, and where more profitable results may be obtained should the findings of this work prove useful.

In Boston and its suburbs bakeries of this type are not directly affected by trouble arising from mold infection, because the bread is not over eight hours old when it reaches the centers of distribution. This fact suggests that a high degree of efficiency in distribution may aid materially in reducing or even in preventing the losses caused by mold trouble.

As may be later seen, loaves from these plants which were kept under conditions analagous to those of shipment and sale were without exception found to mold, and it was on such loaves that our work was largely done.

With the ordinary local bakeries the entire output is small, and the overturn of the product is so rapid that mold trouble, except in a few rare instances, is not known on their own premises, and in these few instances the loss is so insignificant that the proprietors of these establishments have little interest in its prevention. However, the fact remains that any bread as handled at the present time will mold and its time of molding may be longer or shorter according to a number of conditions, and it is with a knowledge of these facts that the following work is presented.

Studies here presented were confined to one type of loaf. This loaf is made from a sponge process dough weighing 1½ pounds and weighs 1 pound 5 ounces when baked. It is baked in Duhrkop ovens for a period of about 32 to 35 minutes.

Review of Literature

A review of the literature bearing upon this subject revealed the fact that very little work had been published. In English, a few articles were found, but this work was not very extensive; in German, the articles showed a much more exhaustive and complete study.

The work of Dr. W. Herter and Dr. A. Fornet, which appeared in the Centralblatt fuer Bacteriologie, 1919, entitled "Studies on the Molding of Bread," treated the subject very thoroughly. The following is a resume of their work:

Eleven molds were found to be present upon bread, as follows, listed in the order of the frequency of their appearance:

Aspergillus glaucus, Rhizopus nigricans, Penicillium crustaceum, Oospora variabilis, Penicillium olivaceum, Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Aspergillus nidulans, Aspergillus condidus, Mucor pusillus.

The molds enter the bakery by means of the flour; the flour, in turn, by careless handling, etc., infects the air and utensils, which finally infect the bread.

The factors concerned in the molding of bread are:

Moisture (of the substrate and the surroundings),
Warmth,
Sugar,
Acidity, and
Oxygen.

The molds which grew most rapidly were Rhizopus nigricans and Mucor pusillus. Penicillium crustaceum was the slowest growing mold. Aspergillus glaucus will grow with the least moisture.

The thermal relations were investigated by the workers and graphs were included, showing the rates and extent of growth of various species of molds at different temperatures.

The effect of moisture upon molding was ascertained by various experiments, as also was the effect of heat. Moist air and high temperatures favor mold growth; bread molds quicker in the summer than in the winter.

Experiments to prevent or inhibit molding by the use of salicylic acid and also by changing the grade of flour and yeast, have proved fruitless.

The article contained a complete resume of the literature concerning this subject, though they found nothing in it relating to the most frequent types, at what temperature they appear, etc. The following is a resume of the literature, listing briefly the work carried out by each investigator.

WELTE—Welte was the first worker upon this subject. He treated especially the effect of molding on the chemical composition of bread. He worked with Penicillium glaucus, Aspergillus nidulans and Rhizopus nigricans. The identification of species was uncertain, although great care was taken; no pure cultures could be obtained.

HERTER—Herter held similar views to the preceding and following worker. He carried out several experiments showing on what kind of bread the molds have been found.

WEHMER—Wehmer found Aspergillus glaucus chiefly upon black bread.

LAFAR—Lafar sifted the literature upon the subject, and found chiefly Penicillium glaucus, Aspergillus glaucus and Rhizopus nigricans.

LINDAU—Lindau found Monilia aurea.

MURTFELD—Murtfeld, in 1917, found Oidium aurontica.

LINDNER—Lindner found Oospora variabilis.

The changes taking place in bread, due to molding, have been much investigated. It has been found that the nitrogen increased slightly and that the carbohydrate was much used up. Peptones were not found while albumins were changed into partly soluble nitrogen compounds. Welte states that moldy bread is not harmful and produces no toxic effect upon animals; the taste is not bitter. To prove this point, he drank a solution of moldy bread and ate some rolls spread with Penicillium crustaceum.

The importance of preventing mold growth on bread is, therefore, not that it produces a toxic effect, but that it is important from the bacteriological standpoint; bacteriosis goes hand in hand with mycosis. Of course, it is also important from an economic view.

The literature, reviewed upon this subject, was chiefly concerned with the types of mold found on bread. There are many illuminating articles listing the types of mold already found, and some, including new groups, hitherto not described.

However, there has been no work found which treats systematically the source and method of infection, or which outlines definite preventive measures against the trouble. The work of Herter and Fornet is perhaps the most complete in this respect, but they lay special emphasis on the studies of molds occurring on bread.

Sanitary Surveys

In order to determine the possible relation between sanitary conditions and the prevalence of molding, sanitary surveys of a number of bakeries in Greater Boston have been made.

In undertaking this portion of the investigation, a number of bakeries that would be representative of the diverse conditions likely to be encountered in the industry was selected. Through general acquaintance with several types of bakeries that seemed to be representative, and by the simple expedient of consulting the telephone directory and making a reconnaisance of the places listed, it was possible to locate a number of concerns suitable for our purpose. The types selected, nine in all, were (1) a "system" bakery; (2) an Italian cellar bakery; (3) an Italian model bakery; (4) a suburban American bakery; (5) a "system" bakery; (6) a large modern scientific bakery; (7) a smaller scientific bakery; (8) a small city-store bakery; (9) an inferior French city bakery.

A sanitary survey was made, covering all the points listed in the score card, and the bakeries rated on a basis of 100% as perfect. Some difficulty was experienced in rating the different establishments, due to almost negligible shades of incongruity when comparisons of the points in question were made. But by assuming an absolutely unbiased attitude and by constantly revising the different scores assigned, what is believed to be a fairly accurate decision in all cases was reached. The scores are appended. A brief description of each may be useful to students.

(1) A "system" bakery, was located in the heart of a business section. On one side was a quick lunch room, on the other a tobacconist. The rear of this bakery gives on a dirty court yard, moderately strewn with rubbish. Here nearly twenty refuse cans, containing refuse material from the lunch room, next door, stand exposed to flies—except in the case of those few cans which are adequately covered. A sewage drain in this court yard is located nearly at the back door of the bakery and, while it was indeterminable as to whether or not it is systematically oiled to prevent nuisance, it gave off an offensive odor on the day examined. The shop itself was above ground, fairly well lighted and mechanically ventilated. Doors were screened, windows kept closed and the shop surprisingly free from flies, considering the obvious fly nuisance presented by the insanitary condition in the court yard behind the shop.

Walls and ceilings were below par in cleanliness, the walls being of corrugated metal, whose paint coat was nearly covered by a fine settling of dust and flour particles. The floor was of wood, very clean, well kept and water tight. No water closet nuisance was observed, but a sink, exposed and rather dirty, for general washing purposes, was exposed in the open rear shop. Plumbing satisfactory.

The employees were neat, uniformed, clean in person and on the whole very excellent. The personnel consisted of four girls, one baker, and one negro worker who was not quite up to the standard of the white help.

Utensils, mixers, dough racks, etc., were only moderately clean. Raw materials were stored in an anteroom, which, though clean, well ventilated and floored with cement, was open to the rest of the shop. Here the materials were poorly covered and on the whole negligently stored. Observations on handling of products during manufacture were only moderately satisfactory, although handling of the final product was perfectly so, except that the bread was wrapped only at time of sale.

(2) An Italian cellar bakery. Poorly lighted and ventilated and representative of the lowest degree of sanitary excellence. Located in a district heavily populated with foreigners and on a street crowded with their shops, clothing stores, etc., typically dirty and congested. This shop has a U-shaped floor plan with one leg of the U abbreviated, thus more resembling a J, and leading to a door beyond which we were not permitted access and beside which leaned a shot gun.

Shop screened but only fairly free from flies, due to their abundance in the vicinity and the constant entering of customers through an ill-fitting screen door. A small basement window on the left side of the shop looks out at ground level upon a filthy alley and while this window is screened, more or less adequately, air currents may bring in dust and dirt. Plumbing exposed and wrapped with rags and a fairly clean sink exposed in rear. Lavatory compartment where sink is located lacks glass in its window frame.

Employees clean, and apparently healthy and noticeably neat in their work, but not neatly nor cleanly clad. All utensils fairly clean, some very much so, and, considering the fact that there is no mechanical equipment, except a mixer, the product is handled very satisfactorily during the process of manufacture. The final product, however, is exposed to flies and handled considerably by customers.

Only one or two ovens was in use and in the firebox of the idle oven, showing that it had not been in use for some time, were several old, hard, moldy loaves of bread. These had apparently been tossed in there to be out of the way. The mold spores from this source might readily be conveyed, by flies or air currents, to infect fresh loaves. Cobwebs were fairly profuse in the far corners of the shop. A barrel of water was standing in rear shop. Flour was stored fairly satisfactorily in the more distant short leg of the U. This part of the shop, however, was moderately dirty and very dark when not artificially illuminated.

(3) A model Italian bakery. Sanitary conditions here, especially in view of the location, which was approximately the same as No. 2, were very excellent. Proprietor and employees were neat, clean, very much interested in our work and courteous and obliging. All equipment was new and the shop installed in a remodeled store where floors, wall sheathing and ceilings were new and freshly painted.

The only objectionable feature, beyond limitations imposed by location and class of trade, was lack of proper screening with its consequent bad exposure to the fly nuisance. This feature, however, was remedied at once by the proprietor as soon as the screens, then already ordered, were delivered to him. Protection of goods before screening the shop was not satisfactory, due to exposure to flies, but now this is entirely satisfactory.

It is notable that, since screening and under conditions otherwise identical, the mold growth on material from this shop is less abundant and less rapid.

(4) A suburban American bakery. The proprietor is a man interested in the application of science and scientific methods to his industry and his interests are in the whole baking industry at large. The establishment is a general bakery and caterer's shop.

Our studies here are previous to the installation of modern mechanical equipment, which the proprietor plans to effect within a year, and since present methods and equipment are rather old, it will be interesting and valuable to check up again on the effect of the new methods and equipment upon mold growth.

Walls were half sheathed and moderately clean; floor sprinkled with sawdust and moderately clean; ceiling very high and clean. Lighting was good and screening excellent. Trays were moderately clean and lined with clean new paper. A sink in the open shop was rather carelessly littered and only moderately clean. Overhead in the middle of the shop were suspended two long planks (of the type used by caterers for banquet tables) which were dusty and on the upper side and might well sift down dust and flour particles of high mold content when heavily laden with such matters and jarred by passing trolleys, trucks, etc., outside. Several clean waste barrels were about. Mixing and raising tubs were of wood and old, and the ovens were old coal ovens with the fire in one corner of the hearth, fed from a coal shed which opened into the shop. The entire shop was dusty and in need of mechanical ventilation.

The personnel of the rear shop (the front shop or sales room being separate and perfectly satisfactory, therefore not in need of description) was acceptably clean, neat and uniformed and consisted of four men. Their uniforms on the day of inspection were somewhat soiled.

In the basement were several undesirable conditions. Fruit flies were profuse and obviously breeding rapidly in and about stored and opened tubs of commercial pie filling. Poison fly-paper was exposed. A wooden sink for dish washing, in one corner beneath the cellar stairs, was littered and dirty. A few cobwebs were in evidence, though not excessive for a basement. Two toilets were in this basement, protected by double partition and double doors, whose action, however, was not automatic. The toilets, though dingy and slightly dirty, did not constitute a nuisance, but lack of lavatory facilities was a bad feature.

The rear part of the basement was equipped as an ice cream plant with an excellent, well drained, concrete floor; entire basement well screened and with fair daylight and adequate artificial lighting. Flour stored here is dry and well kept. The rear of the shop opens onto a large court-yard which, observed from the proprietor's delivery platform, was clean, although some miscellaneous rubbish barrels of neighborhood shops and stores were present. These, however, did not constitute a sanitary nuisance.

(5) A "system" bakery on a very busy down town street. Above ground, fairly

well lighted, and with good artificial ventilation. Excellently screened from flies. Floor and ceiling clean and satisfactory, but walls corrugated and covered with dust and flour particles. No water closet nuisance and plumbing satisfactory, and a basin for general washing purposes at rear of shop.

Employes satisfactory, but not quite immaculate in uniform. Personnel consisted of three bakers, three girl clerks, one messenger.

Utensils, mixers, dough troughs, racks, etc., scrupulously clean; raw materials well protected; rear door, though screened, often left open—draft, however, precludes flies.

Basement clean and dry and used for storage, also used for doughnut mixing. Pastry ingredients stored here and flies present, though not numerous, on jam, etc.

On the whole, a very acceptable bakery and it is to be supposed from the system of management, whereby each shop in the system is managed by a woman (and these managers meet often), that competition between managers serves to maintain standards.

(6) A large industrial plant. Local representative of a large baking concern. Perfect in every detail.

(7) Another large plant, though on somewhat smaller scale than No. 6. The only criticism is on their shipping department. The shipping room opens into the wrapping room and air currents enter from loading platform outside. This feature becomes more objectionable by virtue of use of horse and wagon delivery instead of trucks, making the loading shed objectionable from sanitary standpoint.

(8) A small city store bakery. Located on a main street but rather on the outskirts of the business center. The establishment consists of store and shop. Both above ground and fairly well lighted, but open windows on street level permitted the entrance of dust through ineffective screens.

Ventilation is accomplished by windows and fairly well carried out. There was evidence of flies and cockroaches. Walls and ceilings were whitewashed, but showed accumulation of dust, while the floor was untidy and of an absorptive material.

The employees were of average appearance and the equipment was half modern and half old-fashioned.

The cellar where flour is stored was damp, but quite clean; while such other materials as lard were kept in uncovered receptacles.

The handling of materials in the process of manufacture was not particularly hygienic. The mixing was done by hand in wooden side-wall troughs.

The final product was placed on sale in the store, exposed to dust and flies and not wrapped.

(9) An inferior French city bakery. This shop is located in the heart of the business district. The establishment itself consists of two rooms, store and shop.

The ceilings are covered with sheet tin and the walls plastered. Both are painted white but not overly clean. The floors are wooden and as a rule fairly clean.

Ventilation is by means of a fan in the ceiling which opens on a court. Lighting is by electricity, there being no windows in the shop proper.

Mixing is done by machines, but all other processes are by hand. Proofing is carried out in open wooden trays. The bread is baked in ovens without steam, for fifteen minutes at 425° F.

Yeast and flour are stored in the cellar which is very dirty and has a foul odor.

Toilet is over the shop and connects by means of stairway.

The employees, four in number, are not overzealous in their personal care, all wear caps and work in shirts.

Bread is cooled in wooden barrels with no precautions taken to prevent contamination from the air which is very dusty.

On the second of September, 1921, a general survey to ascertain the relative humidities of the different establishments was made with the following results:

	Humidity Degrees Fahrenheit	Temperature Degrees Fahrenheit
No. 1 in the workroom......	60.4	86
No. 2 in the workroom......	69.2	83
No. 3 in the workroom......	63.4	86
No. 4 in the workroom......	55.0	..
No. 5 in the workroom......	61.2	88
No. 5 in the basement.......	64.8	82
No. 6 in the sponge room....	65.6	84
No. 6 in the mixing room....	68.4	84.3
No. 6 in the dough room.....	64.6	84.4
No. 6 in the conveyor room..	65.4	85
No. 6 in the making up room	65.2	85.2
No. 8 in the oven room......	54.4	89
No. 6 in the shipping room...	52.1	91.5
No. 6 in the basement.......	57.2	87
No. 8 in the workroom.......	65.6	84
No. 9 in the workroom......	53.4	91

These results were obtained using a wet and dry bulb thermometer, degrees Fahrenheit. A graph has been made to show the divergence between shops and even within shops on a given day and under practically the same conditions.

Name Bakery ....Date ....

Address ....Remarks ....

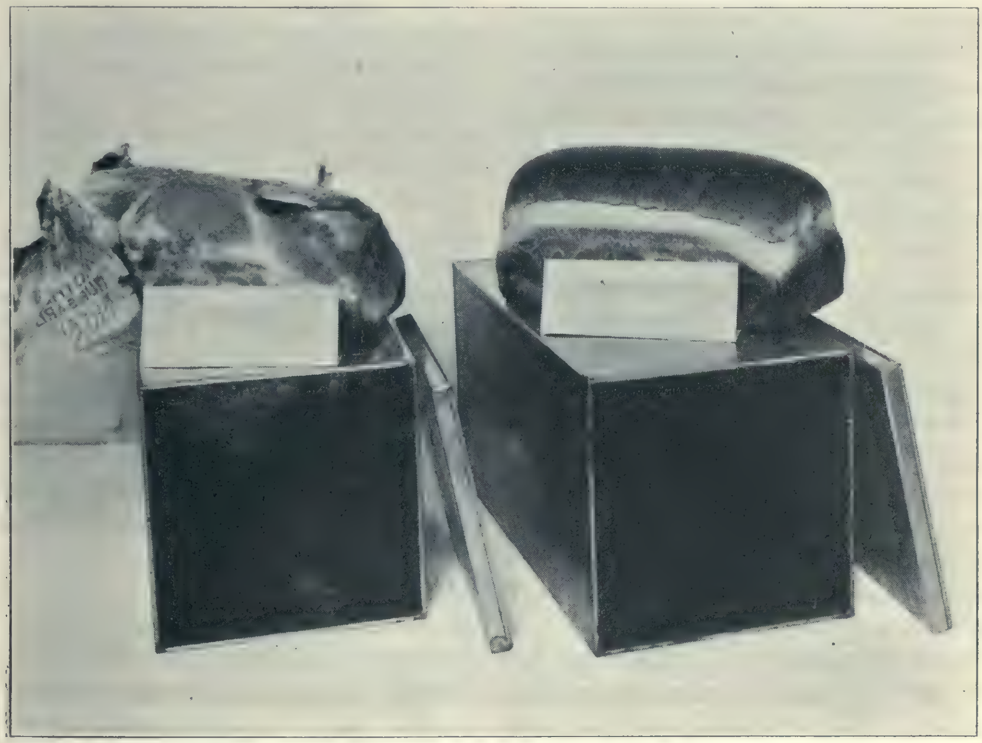
	Perfect	Allow								
		1	2	3	4	5	6	7	8	9
1. Above ground	10	10	0	10	7	10	10	10	10	10
2. Well lighted	10	7	2	8	7	8	10	10	9	7
3. Well ventilated (artificial).............	5	5	0	3	3	5	5	5	3	5
4. Unconnected by door or hall with living room or room used for other business..	5	5	2	5	5	5	5	5	5	5
5. Screen, 2; free from flies, 3..............	5	3	2	3	5	5	5	5	2	4
6. Floor, walls and ceilings clean (deduct 5 for spitting on floor)................	10	4	2	10	6	7	10	8	5	4
7. Water-tight floors	5	5	5	5	5	5	5	5	4	5
8. Water closet does not open into room in which food is handled................	5	5	5	3	4	5	5	5	5	5
9. Basin for washing hands, sanitary plumbing	5	2	3	4	4	4	5	5	4	4
10. Employees wear clean uniforms, 2; free from disease, 3......................	5	4	3	5	4	4	5	5	4	3
11. Utensils, mixers, dough troughs and racks clean	10	6	7	10	8	10	10	10	7	6
12. Protection of raw materials.............	5	2	1	4	3	4	5	5	4	4
13. Handling of products in manufacturing...	5	3	4	5	4	4	5	5	2	2
14. Mechanical mixer	5	5	5	5	0	5	5	5	0	0
15. Handling final product..................	5	4	2	4	4	4	5	2	3	3
16. Bread wrapped in bakery................	5	0	0	0	0	0	5	4	0	0
Total	100	70	43	84	70	85	100	94	67	67

INFECTION DUE TO INGREDIENTS

Believing that the primary source of mold infection lay in the ingredients used in the manufacture of bread, steps were taken to examine these ingredients as possible carriers of mold spores. Flour, being the ingredient used in largest amounts, received the most attention along these lines, but yeast was also included along with other materials. It may be stated at this point that practically every ingredient of bread, with the exception, perhaps, of the shortening, is likely to contain large numbers of microorganisms, and especially bacteria and mold.

In studying the yeast, the cakes were diluted in sterile water and, using wort agar and the plate and platelet technique, the samples were permitted to germinate. The platelet method is used in obtaining pure cultures and consists of placing drops of the medium on the sterile dish instead of pouring the whole plate. On the whole, yeast is not badly infected with mold. These experiments showed molds to be present but not to the extent of any heavy infection, such as is found in flours.

The flours were examined in the following manner: Samples of the flour were placed in sterile Petri dishes, and mixed with sterile water, using a sterile spatula. When thoroughly mixed, the plates were kept at room temperature and observed from day to day. The reaction taking place in a typical flour, when treated in this manner, is somewhat as follows: First day, no change; second day, the mixture has a cheesy or yeasty odor, and gas bubbles demonstrate that fermentation is in progress. The mass is yellowish orange in color. The beginnings of fruitless mycelia are observed. Under the microscope yeast cells were evident and Bacterium lactis was thought to have been observed, although not verified; third day, the sample has a cheesy, rancid odor, black and green molds (Rhizopus and Aspergillus) in evidence and red



STERILITY VERSUS EXPOSURE TO MOLDS

A loaf when sealed against mold spores remained fresh for three weeks.

spots and yellow spots develop; fourth day, the plate has a putrid, musty odor and is practically covered with fungus growth.

Ten different samples of flour were examined in this way and all showed heavy infection with molds. The types most frequently encountered were Rhizopus nigricans, Aspergillus glaucum, Aspergillus ostianus, Aspergillus candidus, and Penicillium glaucum.

The gluten was separated from the flour and inoculated with mold and the remaining portion of the flour treated likewise. The growth on gluten was slow, and not characteristic, while the growth on the starchy portion was heavy and normal.

Heat treatment was tried in an effort to sterilize the flours. Flours subjected to dry heat at 160-170° C. for a period of one hour became dextrinized. Penicillium glaucum, when present, survived. Trying a lower temperature for a longer time, flours were heated at 130-140° C. for 85 minutes. These did not dextrinize, but as before, Penicillium glaucum survived. This work and subsequent work showed that sterilization of flours is impractical, and, as shown later, unnecessary.

EXTENT OF INFECTION OF LOAVES

To determine the extent of infection on loaves offered for sale, it was decided that the best and most obvious method would be to permit such loaves to mold spontaneously. Accordingly, loaves were collected from different bakeries as they were about to be sold, and taken immediately to the laboratory. Some of these loaves were placed under sterile bell jars in an atmosphere highly saturated with water vapor so as to furnish the optimum conditions for the development of molds. They were also shielded from the light, as this is another factor which influences the growth of mold fungi.

During the summer months the mold colonies appeared on all loaves within two or three days. The amount of infection was approximately the same in all cases where the degree of sanitary excellence was nearly on a par, but those bakeries having a low degree of sanitary excellence showed a heavier infection. The infection in summer was heavier than in winter. This is due to the greater amounts of dust, fly nuisance, and method of handling, as well as other factors. In winter the development of molds is slower, oftentimes a period of six days elapsing before the first appearance of mold structure.

Plate exposures were made at various points in the different plants (using a 2% dextrose agar which was found to be best for mold culture) in order to ascertain the extent of infection due to the air, and to discover the correlation, if any, between types carried in the air, and the types appearing on the loaves.

The results of these experiments showed that the types in the air and the types on the exterior of the loaves were identical. The types (genera) are Aspergilli, Penicillium, Rhizopus, and Mucor. Experience showed that Mucor occurred very rarely in winter, whereas it was frequently encountered during the summer work, Rhizopus and Aspergillus being met most often.

A phase of the work which afterwards proved fruitless in the light of later work, was the examination of different portions of the individual loaf. Loaves were collected and different portions such as "center of loaf," "side center," "under crust" and "crust" were incubated in sterile Petri dishes with sufficient sterile water to encourage the generation of molds. It was found that without exception all of these portions developed molds. However, examination of the loaf at the center, the exterior of which was completely covered with mold, showed no mold growth. The mold seemed to start at the exterior and by a process of ingression of the hyphae advance toward the center. There was no initial growth at the center. But the plated samples of the centers of loaves showed infection of the centers. These two results were conflicting and at variance with each other. However, the plated samples may have been open to infection through faulty technique or other source, whereas the unopened loaves left no such possibility for contamination. The point to be determined, then, was the completeness of sterilization of the loaf by the baking process, and steps were taken to determine this point.

STERILITY OF THE BAKED LOAF

On the determination of this point, more time was allotted, perhaps, than to any other one phase of the investigation, because it was deemed of such importance that a decisive conclusion on this point must be reached before going further with the work.

Loaves were collected directly after they left the oven, and, handling with aseptic precautions, were placed in sterile tin containers especially constructed for this purpose. These loaves have been kept for varying periods under these conditions and even at the end of three weeks the loaves showed no evidence of mold infection or growth on the exterior or in the inner portions.

Other loaves were collected and handled in the same manner but on reaching the laboratory they were coated with a film of paraffin by dipping in melted paraffin at 55° C. At the end of three weeks the coating was cracked off and the loaf exposed with the following observations:

There was no evidence of mold infection in any part of the loaf.

There was very little loss of moisture, the bread being very palatable.

The taste was practically normal, though a slight acid taste was noticed.

Inoculations into litmus milk gave coagulation with acid. (See later for bacteria).

There follows some of the data from the experiments performed to ascertain the sterility of the loaf.

1. Two loaves were collected in sterile containers and kept for five days.

External appearance—no mold growth
Internal appearance—no mold growth

Six portions of the crumb were placed with aseptic precautions in sterile Petri dishes and sufficient sterile water added to make mold growth favorable.

1 day—no mold growth
2 days—no mold growth
3 days—no mold growth
4 days—no mold growth
5 days—1 plate showed Rhizopus
6 days—2 plates showed Rhizopus

2. Four loaves were collected as above and examined at the end of four days.

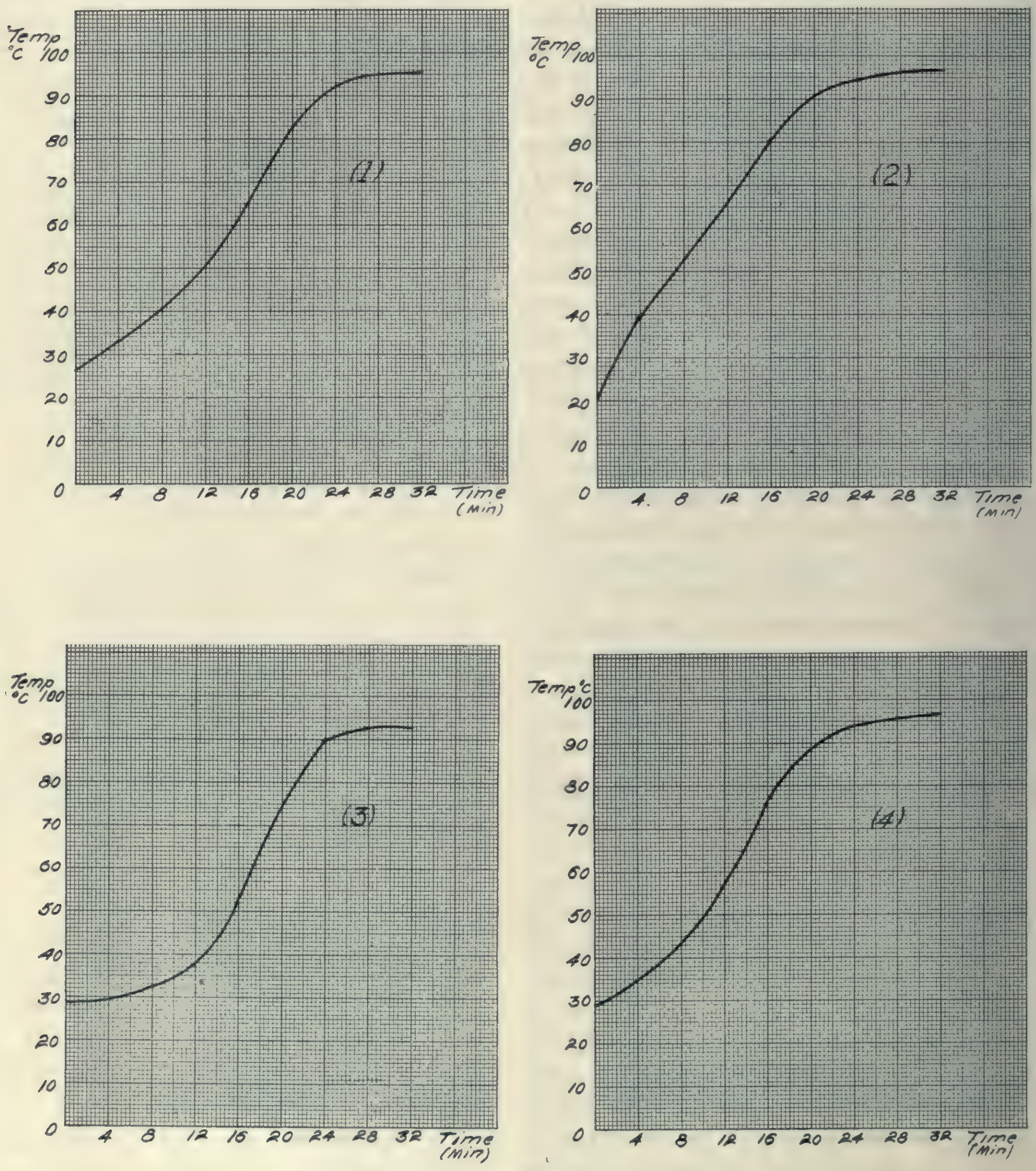
External appearance—no mold growth
Internal appearance—no mold growth

The centers of these loaves were plated in sterile Petri dishes as before, 20 plates being made.

24 hours—no mold growth
48 hours—no mold growth
72 hours—3 plates showed Rhizopus
96 hours—4 plates showed Rhizopus
120 hours—5 plates showed Rhizopus
144 hours—5 plates showed Rhizopus

The plates showing mold growth were not from the same loaf.

The appearance of mold growth on these plates was viewed with suspicion. It could not be decided whether this mold had survived the baking temperature or appeared on the plates due to faulty technique, since the laboratory itself had previously given evidence of infection of the air by molds to quite a large extent.



RISE IN TEMPERATURE IN THE LOAF.

Graphs showing how temperature increases during baking until it exceeds that at which mold spores can live.

Accordingly, steps were taken to decide these facts, and as a matter of general information, the temperatures at the center of a loaf during baking were determined by thermo-couple measurements.

3. The procedure followed in taking thermo-couple measurements of the centers of loaves was worked out along the usual methods involved in this type of temperature work.

The hot junction was inserted in a glass tube which was sealed at one end and bent almost at a right angle. The closed end was inserted in the dough so that the junction was approximately at the center of the loaf, and that left of the angle parallel to the bottom of the pan. The object attained in bending the tube was to insure a larger portion of the junction being in more exact proximity with the center.

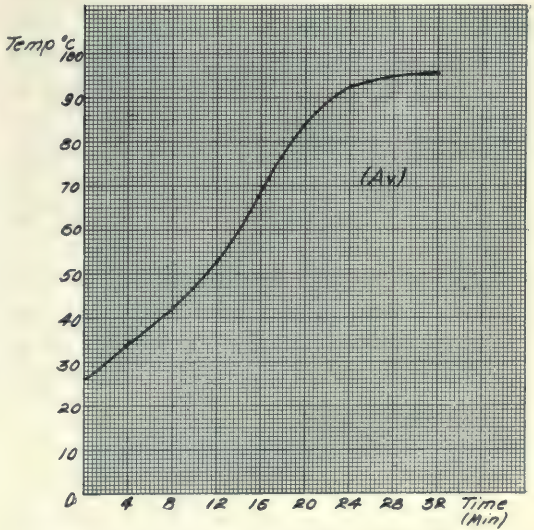
The pan was then put into the oven, the leads being taken out through the door in asbestos coverings. The cold junction was an uninsulated bottle containing water and stoppered with a rubber stopper perforated by a centigrade thermometer. The readings were taken from the scale of a millivoltmeter. For the purpose of refreshing the fundamental facts concerning the thermo-couple, it might be well to give a brief explanation here.

When two different metals are joined together in a circuit, and one junction is heated, an electromotive force is in general produced which tends to drive a current in a certain direction, and this electromotive force increases as the difference in temperature between the two junctions increases. A millivoltmeter suffices to measure the electromotive force generated by heating one junction, and it may be calibrated to read temperature directly. The thermo-couple is valuable on account of its sensibility, quick response to temperature changes, and the small size and mass of the part which must be heated as compared to the bulb of a mercury or a resistance thermometer.

The following data shows the temperatures of the four best runs of a series made on a sponge process loaf which, baked, weighs one pound five ounces, and is made from 1½ pounds of dough. It is baked in Durkhop ovens in the front row and the oven temperatures ranged between 30° F. and 420° F.

Time				Temp.	°C.
Min.	1	2	3	4	Av.
0	26.5	20.5	28.8	29.0	26.2
4	33.2	39.5	29.3	34.8	34.2
8	40.5	52.5	32.5	43.0	42.1
12	50.2	66.0	37.3	57.0	52.6
16	65.5	80.7	53.1	76.5	68.9
20	82.5	91.0	74.5	88.7	84.2
24	92.2	94.3	89.5	94.0	92.5
28	95.0	96.4	92.4	95.5	94.8
32	95.5	96.7	92.7	96.8	95.4

Maximum thermometers placed in the centers to check the final readings registered 96.5° C. and 97.0° C. respectively.



Average temperature at which loaves of four bakes exceeded the death point for mold spores.

The following technique was worked out and used in connection with the problems of the sterilization by the baking process.

Into sterile Erlenmeyer flasks containing 10 cc. of 1% NaCl solution, sufficient flour was added to stimulate the consistency of dough as it enters the oven. These flasks were heavily inoculated with pure cultures of molds of the general Penicillium, Aspergillus, and Rhizopus types and plugged with sterile cotton plugs.

The flasks were immersed in water at about 20° C. and, following the curve obtained by thermo-couple measurements of the temperatures at the center of the loaf, as nearly as possible by controlling the temperature, the water was brought up to boiling in a period of 35 minutes. The flasks were permitted to cool over night in the water, and were then incubated at 37° C. to permit the development of any spores that might have survived.

Result: No mold growth was obtained in any of the flasks, showing that no mold spores had survived the treatment.

4. Since Rhizopus was the only mold that appeared on any of the plates containing the crumb of the loaves in the foregoing experiments, the following study of temperatures at which this mold ceases to live was made. The baking temperatures at the center of the loaf as previously determined, reach 97° C. and are above 80° C. for 12 minutes or longer.

The death point determinations on Rhizopus were made by inoculating the mold on sterilized bread cut from the center of a loaf. The bread was of such size as to fit into a Petri dish and was then sterilized in the autoclave at 15 pounds for 20 minutes. The table below shows the results:

Temp. °C.	1 d.	2 ds.	3 ds.	4 ds.	5 ds.	6 ds.
105	—	—	—	—	—	—
100	—	—	—	—	—	—
95	—	—	—	—	—	—
90	—	—	—	—	—	—
85	—	—	—	—	—	—
80	—	—	—	—	—	—

Three controls (no heat treatment) showed abundant growth of Rhizopus.

5. Three loaves were heavily inoculated with Rhizopus before being placed in the oven. They were collected and maintained under sterile conditions in containers.

At the end of five days one loaf was examined. There was no evidence of mold growth on the exterior. That portion of the center containing the inoculation was plated in six Petri dishes and sufficent sterile water added so that, should there be any live spores, favorable conditions for growth would be present. Results:

2 days—no growth
3 days—no growth
4 days—no growth
5 days—no growth
6 days—no growth
7 days—no growth

At the end of seven days a second loaf was treated as above. Results:

2 days—no growth
3 days—1 plate showed Rhizopus
4 days—1 plate showed Rhizopus
5 days—1 plate showed Rhizopus
6 days—1 plate showed Rhizopus
7 days—1 plate showed Rhizopus

At the end of two weeks the third loaf was examined as those before. Results:

1 day —no growth
2 days—no growth
3 days—no growth
4 days—no growth
5 days—no growth
6 days—no growth
7 days—no growth
8 days—no growth

This series of experiments proved conclusively that mold spores do not survive the baking temperatures and that the rare cases in which the presence of Rhizopus was observed are due to outside infection due to the contamination by spores in the air in the process of plating. Thus we believe that the sterility of the baked loaf, as concerns mold, seems to be an established fact.

THE SOURCE AND METHOD OF INFECTION

Since the baked loaf of bread is sterile, the infection takes place after the bread leaves the oven. The possible sources of infection are:

A. During the process of cooling.

B. Contact with the wrapping paper

A. To determine the extent of infection which take place during the cooling process, samples were collected from the bread racks on which they had been exposed to the air. Some were collected in sterile tin containers (autoclave 20 minutes at 15 pounds pressure); others were collected in sterile glass containers (sterilized in the same manner). The samples were collected at varying intervals of time during the cooling process to determine the relation between the time of exposure of the loaves to the air and the extent of infection.

Several loaves, which had cooled for varying periods of time, were wrapped as customary. This was done to ascertain how soon after leaving the oven it is safe to wrap the loaf, so that it does not sweat. This latter experiment shows the infection due to the paper as well as that due to infection received during the cooling process.

1. Two loaves—cooled 15 minutes.
   One loaf was placed in a sterile glass container; the other was wrapped and placed in a sterile tin container.
2. Same as 1—cooled 20 minutes.
3. Same as 1—cooled 5 minutes.
4. Same as 1—cooled 30 minutes.

Result:

1. Loaf in glass container.

No growth up to seventh day
7th day—appearance of green mold (Penicillium, white Mycelium, no fruiting)
8th day—appearance of green mold (Penicillium, Aspergillus)
9th day—appearance of green mold (Penicillium)
10th day—appearance of green mold (Penicillium)
11th day—appearance of green mold (Penicillium)
12th day—appearance of green mold (Penicillium)

The wrapped loaf in tin container was examined at the end of twelve days.

Appearance:

Abundant mold growth. The types present were Rhizopus and Aspergillus.

Rhizopus was present on this loaf and not on the previous loaf—may have come from paper, not necessarily, however.

2. Same result as obtained in 1, though the infection was slightly heavier. Glass container.

No growth up to seventh day.
7th day—appearance of greenish mold and white mycelial growth.
8th day—appearance of Penicillium and Aspergillus
9th day—appearance of Penicillium and Aspergillus
10th day—appearance of Penicillium and Aspergillus
11th day—appearance of Penicillium and Aspergillus
12th day—maximum growth.

Wrapped loaf showed Rhizopus in addition to the above types. This loaf showed sweating; thus, 20 minutes is too soon to wrap the loaf after it leaves the oven.

3. Glass container.

No mold growth until the 7th day
7th day—white mycelial (no fruiting structures)
8th day—Aspergillus
10th day—Aspergillus
11th day—Aspergillus
12th day—Maximum growth

Loaf could not be wrapped at end of five minutes. The loaf in this case showed only one type of mold and the growth was small; very much less abundant than in the previous examples.

4. Same result was obtained as in the two preceding cases, except that the loaf showed growth of Rhizopus in addition to Penicillium and Aspergillus. The wrapped loaf in the tin container showed the same types of mold as the unwrapped.

CONCLUSION:

Loaves become more or less heavily infected with mold spores during the process of cooling. The amount of infection increases as the time of cooling increased.

The types of molds found on the loaves which have been cooled, but not wrapped, were: Rhizopus, Penicillium and Aspergillus. Thirty minutes is the minimum time in which a loaf can be wrapped.

Several experiments were carried out to determine the extent of infection of the loaf which had cooled and was ready for wrapping. Several samples were also tested to determine the additional infection of those loaves due to wrapping.

1. Two loaves which had cooled 30 minutes were placed in sterile tin containers. They were examined at the end of 12 days so as to allow for maximum growth.

Appearance—12 days
Exterior of the loaf was entirely covered with mold growth.

Types—
Aspergillus (3)
Penicillium

2. Two loaves had cooled 30 minutes were wrapped and placed in sterile tin containers. These loaves were examined at the end of 12 days.

Appearance—
Very abundant growth of mold. Exterior of loaf entirely covered; mycelia had penetrated in wrapper.

Types—
Aspergillus (2)
Penicillium (2)
Mucor } Did not occur in previous case (un-
Monilia } wrapped loaf); may have been due to paper. See experiments on paper.

POSSIBLE SOURCES OF INFECTION

Sterile Petri dishes containing sterilized dextrose agar media were exposed at various points. This was done to determine the source of infection and to ascertain whether the types found checked with the types found on the bread.

Plates were exposed on the bread racks for the same period of time as the bread is while cooling. They were also exposed on the endless oven belt, wrapping machine belt and the bread handling cloths were tapped over the plates; the plates were placed upright and reversed. After exposure, they were taken to the laboratory and incubated at room temperature.

The following results were obtained:

Bread racks—types:
Rhizopus
Penicillium

Oven belt—types:
Rhizopus
Aspergillus
Penicillium (2)

Bread Handling Cloths—types:
Rhizopus
Penicillium

Wrapping Machine Belt—types:
Rhizopus
Penicillium
Aspergillus

Air—types:
Rhizopus
Penicillium
Aspergillus

The experiment was repeated.

The molds found by making these plate exposures checked very well with the types found on the bread. Thus—

Molds appearing—

On bread	On plates (Petri dishes)
Penicillium	Rhizopus
Rhizopus	Penicillium
Aspergillus (2)	Dematium pullulans
	Aspergillus (2)

CONCLUSION:

The air, endless belts, etc., are infected with mold spores and serve as the sources of infection of the bread during the cooling process. Thus, from the time the bread leaves the oven until it is wrapped it is constantly open to infection.

Whether or not the paper used for wrapping adds to the infection already existing on the bread is now a matter for consideration.

B. EXAMINATION OF WRAPPING PAPER.

Examination was made of the paper at the bakery to determine the presence of mold spores. Samples were collected from original wrapped rolls and unwrapped and partially used rolls of paper. The paper was collected by means of sterile rubber gloves and placed in sterile copper containers. Small portions of this paper were cut (by means of sterile scissors and forceps) and placed in sterile Petri dishes. Dextrose agar media was added.

Five portions, collected from the wrapped roll, were plated in the method described above, and incubated at room temperature. Observations:

1st day—no mold growth
2nd day—no mold growth
3rd day—no mold growth
4th day—1 plate showed mold growth
5th day—2 plates showed mold growth
6th day—3 plates showed mold growth
7th day—3 plates showed mold growth
8th day—3 plates showed mold growth
9th day—3 plates showed mold growth

Five portions from an unwrapped roll were examined. Observations:

1st day—no mold growth
2nd day—no mold growth
3rd day—1 plate showed mold growth
4th day—2 plates showed mold growth
5th day—2 plates showed mold growth
6th day—2 plates showed mold growth
7th day—2 plates showed mold growth
8th day—3 plates showed mold growth

Six portions of paper collected from the wrapping machine were plated. Observations:

1st day—no mold growth
2nd day—no mold growth
3rd day—no mold growth
4th day—4 plates showed mold growth
5th day—5 plates showed mold growth
6th day—5 plates showed mold growth
7th day—5 plates showed mold growth
8th day—5 plates showed mold growth
9th day—5 plates showed mold growth

The types of molds occurring on the paper were very much the same as the types found present in the air and on the machines, etc., by plate exposures. Thus: Molds occuring—

On the wrapping paper	**On the plates (Petri dishes)**
Penicillium (2)	Rhizopus
Rhizopus	Penicillium
Aspergillus	Aspergillus (2)
Monilia	Dematium pullulans
Mucor	Monilia

The above experiments show that the bread wrapping paper is infected with mold spores. The degree of infection seems to vary with different samples of paper; the paper collected from the wrapping machines showing the heaviest infection. The results obtained above would indicate that the air is an important factor in connection with the infection of the paper, paper which has been longest exposed in contact with the air showing a greater amount of infection. These experiments showed that theoretically the paper contained on its surface mold spores; whether or not these spores would infect the bread and thus cause mold growth thereon was next determined. It must, however, be borne in mind that the molds mentioned are common forms, and that occasionally to find small numbers of these would not be surprising.

2. Two loaves were collected in sterile tin containers immediately upon leaving the oven and were cooled in this manner, thus maintaining their sterility. The loaves were then put through the wrapping machine, after having cooled for a sufficiently long period (30 minutes) and the wrapped loaves were finally placed in sterile tin containers. The loaves were examined at the end of seven days and showed an abundant growth of mold; the only type present was Aspergillus. The paper used to wrap the loaves in this experiment had been exposed to the air for a rather long period of time and had thus had ample opportunity to become infected with mold spores from this important source. The above experiment was repeated, using the same conditions except that the paper, which was used to wrap the loaves, was taken from the inside of the roll; thus the paper used in this experiment had less opportunity of becoming infected from the air than did the paper used in the preceding experiment. Two loaves were cooled, using aseptic precautions and were wrapped as above and placed in sterile tin containers. One loaf was examined at the end of seven days and was found to be covered with a potent growth of Rhizopus while an incipient growth of Penicillium was observed. The second loaf was examined at the end of fourteen days and was found to be practically sterile; one small colony of Aspergillus was found present. These results would seem to indicate that the infection of the paper from the air is rather important and that the paper at no time is absolutely free from mold spores.

3. To follow this matter to the source, a visit was made to the factory in which bread-wrapping paper is made, and a careful examination of the process was conducted.

Bread wrappers are, for the most part, divided into two classes, (a) Dry waxed, and (b) Self-sealing.

DRY WAX BREAD WRAPPERS.

Dry wax wrappers are so called because they carry practically no wax on their surface. A relatively small amount of wax is used in their manufacture and this wax is driven into the fabric of the paper without obtaining the shiny surface coating. Wrappers of this type used on the original Sevigne bread wrapping machine. The machine is relatively expensive but handles a somewhat cheaper paper and wraps at a much higher rate of speed than do any of the other machines. The seams on this wrapper are closed by means of melted paraffin which is applied during the wrapping process from a small reservoir. This method costs more than the self-sealing, but owing to the fact that the paper is more porous, it is perhaps more satisfactory than the latter, as the self-sealing type retains the moisture and produces an inoculated effect, thus promoting mold growth. The self-sealing type, however, keeps the bread from drying out.

The method of waxing the dry waxed grades is to run the raw paper, which may or may not be printed (all printing is done previous to the waxing process) between a steel and rubber roll on the wax machines. The steel roll revolves through the molten wax (212° F.) and carries a certain amount upon the paper. The rubber roll which runs against it serves as a squeeze roll and reduces the amount of wax applied. The web of paper is then run over two or more revolving coppered rolls through which live steam circulates. This application of heat after the wax has been applied serves to drive the wax into the paper; the paper is uniformly impregnated. After leaving the coppered rolls the paper is immediately rolled.

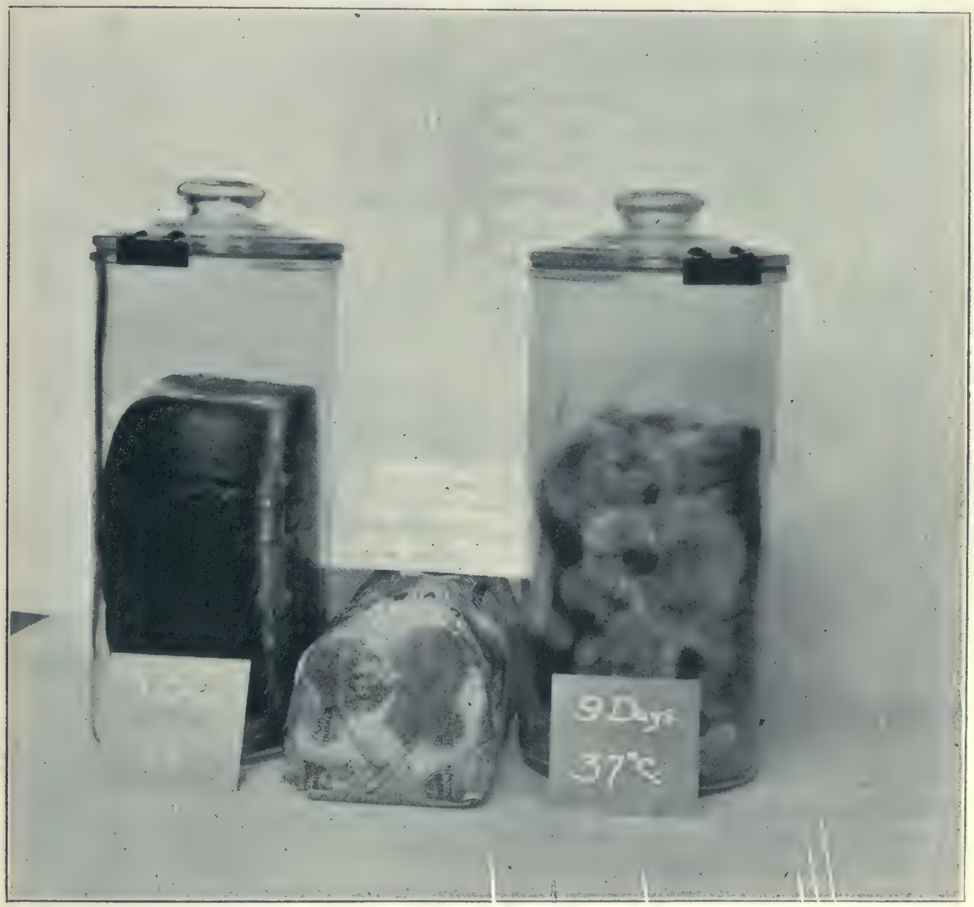
The time of exposure of the paper to the air, and thus to infection, from the time it leaves the coppered rolls until it is wrapped, is about three or four seconds This short interval of time and a similar period when the paper is unrolled, divided lengthwise and rerolled, constitute the sole means of infection. After being rerolled, the paper is wrapped and is ready for shipment.

SELF-SEALING BREAD WRAPPERS.

Self-sealing wrappers are those which contain a sufficient amount of wax in the fabric and on the surface so that when they are wrapped about the bread a seal is effected by passing the loaf so wrapped over a hot plate.

The machines which employ this type of wrapper are the Hajssen and Brownell machines. The machines are slower than the Sevigne, described above.

Owing to the fact that bread wrapped with this type of wrapper retains its moisture longer than the bread wrapped in the dry sheet and also because of the shiny appearance of the surface of the paper, many bakers prefer it to the dry.



EFFECT OF TEMPERATURE ON MOLDS
They grow luxuriantly at 37° C., are halted at 15½° C., and penetrate wrapping paper at 20° C.

The method of applying wax on this grade is to draw the raw paper through the molten wax which is carried at a temperature varying from 180°-210° F. The web is then passed between the steel and rubber rolls as in the case of the dry waxing, which serves to squeeze off the superfluous wax. The paper is then passed over from two to four rolls through which cold water circulates, thus chilling the wax so that the paper can be immediately rolled. The source of infection with this type of paper is the same as with the dry wrap.

From a brief consideration of these processes it is evident that very little infection of the paper is possible in the factory. Samples were collected from the machines treating the paper as the paper was about to be rolled; a sample was also collected from a roll of paper which had been unrolled, divided lengthwise, and rerolled. Petri dishes (containing sterile dextrose agar) were exposed to the air at various points during the process to determine the extent to which the air was infected with mold spores; they were incubated at room temperature.

1. Sample collected from the "dry wrap" machine } about to be rolled
2. Sample collected from the "wet wrap" machine } about to be rolled
3. Sample collected from "dry wrap" (rerolled)

These samples were collected, taking aseptic precautions, and portions were plated in sterile Petri dishes and dextrose agar was added.

1. Five portions were plated.

Observations:

No evidence of mold growth up to the 7th day
7th day— 1 plate showed mold growth
8th day—1 plate showed mold growth
9th day—1 plate showed mold growth
10th day—1 plate showed mold growth
11th day—1 plate showed mold growth; 4 plates sterile.

2. Five portions were plated.

Observations:

No evidence of mold up to 10th day
10th day—1 plate showed mold growth
11th day—4 plates sterile; 1 plate showed mold growth

3. Five portions were plated.

Observations:

No evidence of mold growth up to 5th day
5th day—appearance of mold growth
6th day—appearance of mold growth
7th day—appearance of mold growth
8th day—appearance of mold growth
9th day—appearance of mold growth
10th day—appearance of mold growth
11th day—3 plates sterile; 2 plates showed mold.

Results of plate exposures:

Plate exposures were made near the machines, i. e. (1), (2), (3) above.

(1) Potent growth of mold in 3 days
Types—Penicillium
Aspergillus
(2) Potent growth of mold in 3 days
Types—Penicillium
Aspergillus
(3) Potent growth of mold in 3 days
Types—Penicillium
Rhizopus

Pencillium and Aspergillus species of mold predominated on all plates; Rhizopus was found but rarely and it was not abundant when present.

The plate exposures showed that the air was rather heavily infected with mold spores.

The samples of paper, however, were found to be practically sterile, only now and then a sample showing the presence of fungi. While these results seem not to be in harmony, this discrepancy is apparent rather than real. As a matter of fact the machines run at such speed and the amount of air movement in the mill is so large that there is very little settling of the spores and the infection of the paper at this time is really extremely light. The time of exposure of the paper, as pointed out above, is very limited and so the conclusion may be drawn that the paper as it leaves the factory is practically sterile, though not theoretically so. The amount of infection from the paper mill is extremely small as compared with the infection the paper is exposed to in the more dust-laden atmosphere and machinery of the bakery. Thus, it is convincing that the occurrence of fungi on the paper in the bakery is very largely due to infection actually received at the bakery.

The infection of paper is sporadic, depending on chance; if the spores are present, while the paper is exposed to the air, they will lodge on the paper and infect the bread when it is wrapped.

Paraffin sections of portions of paper from a badly molded loaf, which seemed to show that the mold had grown through the paper, were made. The technique used was as follows:

Sections showing mold on each side of the paper were cut, and the fungus killed in McClenden's fluid. The sections were run through 30%, 70%, 95% and absolute alcohols and then through Xylene. Paraffin blocks were made and the cross-sections cut on the microtome about 10 microns in thickness. These sections were fixed on slides after the manner of the usual histologic procedure for paraffin sections and stained with Delafield's Haematoxylin and mounted in Canada balsam.

The fibres of paper and mycelia were contrasted by the intensity of the stain, and under the microscope, penetration of the paper by the fungus could be seen.

Photomicrographs were taken and are shown herewith.

CONCLUSIONS:

1. Paper as it arrives at the bakery is practically sterile.
2. The paper becomes infected at the bakery.
3. The mycelia of the mold will penetrate the wrapping paper (dry wax type).
4. The types of mold occurring on wrapping paper are the same as those found on infected bread and in the air, etc.

PREVENTION OF THE MOLD INFECTION

In attempting to solve the problem of preventing the molding of bread, there are two possible methods of attacks—(a) by handling in such a manner, after it leaves the oven, that its sterility is maintained; this means that aseptic precautions must be observed thus preventing the mold spores from gaining access to the bread; or, (b) by treating the loaf, which has already become infected, so that the mold spores present are unable to develop. The second method of prevention will be discussed first.

B. The problem of treating the loaf of bread, already infected with mold spores, so that the molding will not take place, resolves itself into the following methods of treatment:

1. Heat treatment
2. Chemical treatment
3. Physical treatment
    a. mechanical coating of loaf
    b. unfavorable conditions for growth of mold (temperature, moisture, etc.).

Experiments were carried out using the various types of treatment listed above. Since the source of infection, as previously determined, was found to be from the air, etc., during the process of cooling and from the bread wrapping paper, loaves were used for the following experiments, which were wrapped and were ready for shipment.

1. HEAT TREATMENT. Several experiments were carried out to determine

the optimum time and temperature for treating the wrapped loaf of bread, so that the growth of mold spores was inhibited. The wrapped loaves were subjected to dry steam in the autoclave and to hot air in the hot air oven; this was done to ascertain which method of treatment is the more efficient. After treatment, the loaves were placed in sterile tin containers, incubated at room temperature, and examined at varying intervals of time. In no case was the interior of the loaf of bread found to show growth of mold.

A. Experiments using autoclave.

Two loaves were exposed to moist air, a temperature of 75° C. for 5 minutes at atmospheric pressure. One loaf was examined at the end of 9 days. The loaf showed the presence of a white mycelial growth with no fruiting structure, showing that the growth was young. There was also found one colony of Pencillium mold and a small growth of Rhizopus.

The second loaf was examined at the end of 14 days. An abundant growth of mold was present, entirely covering the exterior of the loaf. The types found were Aspergillus and Pencillium.

b. Two loaves were subjected to a temperature of 110° C. for 30 seconds at 15 pounds pressure. One loaf was examined at the end of 9 days. The exterior of the loaf showed no evidence of mold growth; the loaf was not reduced in its moisture content and was apparently unchanged.

The second loaf was examined at the end of 14 days. An abundant growth of Rhizopus and Aspergillus mold was present on the exterior of the loaf; the mold mycelia appeared to penetrate the paper.

c. Two loaves were subjected to live steam for 1 minute at atmospheric pressure. One loaf was examined at the end of 7 days; there was no evidence of mold growth present; the loaf was rather moist, but was not unpleasant.

The second loaf was examined at the end of fourteen days. An obvious growth of mold was present; Aspergillus was the only type evident.

4. Two loaves were treated in the same manner as the loaves in (c), except that the time of exposure was increased to 4 minutes. One loaf was examined at the end of 7 days. There was no evidence of mold growth present; the loaf, however, was rather moist and soggy.

The second loaf was examined at the end of 14 days and was found to be practically free from mold, only one small colony of Aspergillus being found.

The loaves treated by means of the autoclave were slightly changed in character; they were moist and soggy after treatment, but after standing from 7 to 14 days they assumed a more agreeable appearance and taste.

EXPERIMENTS WITH HOT AIR (DRY HEAT).

a. Two loaves were subjected to a temperature of 85° C. for 5 minutes. One loaf was examined at the end of 9 days. The exterior of the loaf showed a growth of Aspergillus and Penicillium; the growth was not abundant.

The second loaf was examined at the end of 14 days. A very evident growth of Aspergillus and Penicillium mold was present.

b. Two loaves were subjected to a temperature of 85° C. for 8 minutes. One loaf was examined at the end of 7 days. An incipient growth of Penicillium and Aspergillus was evident. The second loaf was examined at the end of 14 days; a very abundant growth of Penicillium and Aspergillus mold was present.

c. Two loaves were subjected to a temperature of 100° C. for 10 minutes. The first loaf at the end of 7 days showed no evidences of mold growth and no loss of moisture. The second loaf at the end of 14 days showed the presence of Aspergillus and Penicillium; the growth was not abundant and was in the early stages.

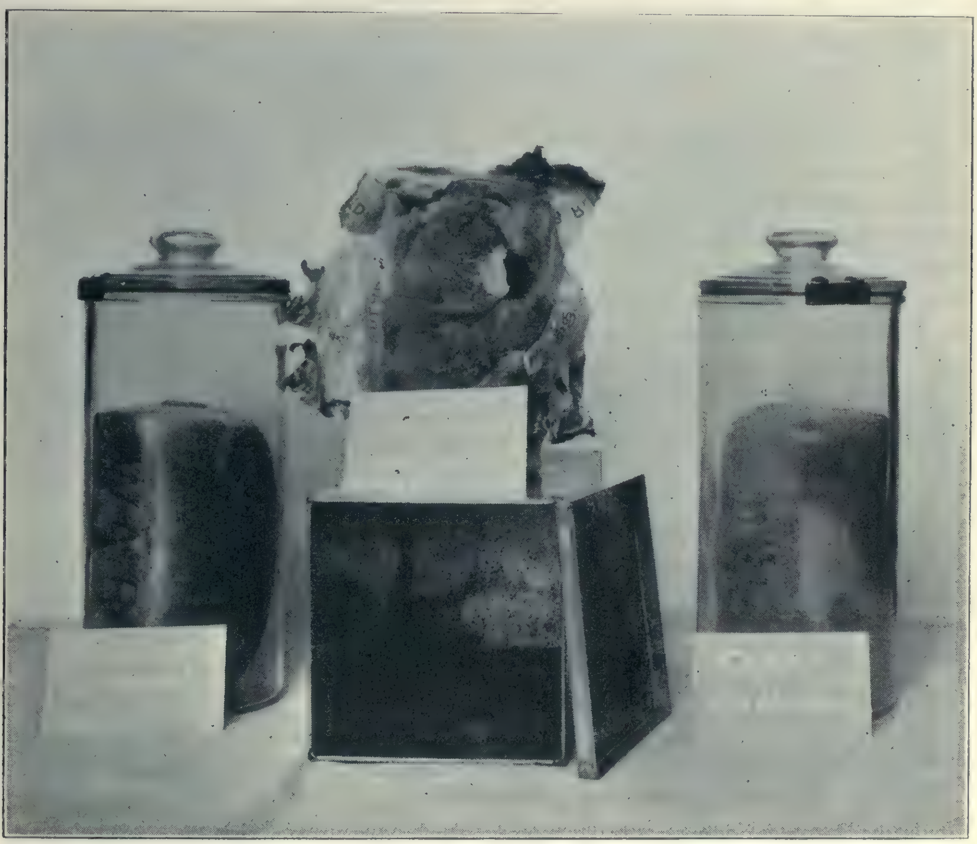
d. Two loaves were subjected to a temperature of 105° C. for 4 minutes. Both loaves were examined at the end of 12 days. There was present an abundant growth of mold on both loaves; the types present were Aspergillus and Penicillium.

e. Two loaves were subjected to a temperature of 120° C. for 5 minutes. One loaf, examined at the end of 7 days, showed a growth of Aspergillus commencing to fruit, the growth was small. The second loaf, at the end of 14 days, showed the presence of one small colony of Penicillium, the growth was very limited.

f. Two loaves were subjected to a temperature of 120° C. for 10 minutes. One loaf at the end of 7 days, showed no evidence of mold growth; there was no loss of moisture. The second loaf, examined at the end of fourteen days, showed the beginning of slight growth of Penicillium and Aspergillus.

Untreated loaves showed mold growth at the end of six days.

The above experiments show that the dry heat treatment is the more desirable of the two methods attempted from the aesthetic standpoint, as well as from the



HOW MOLD GROWTH VARIES WITH TIME

When wrapped warm it increases, and at favorable temperatures expands very rapidly.

standpoint of efficiency. The method of treating the wrapped loaf by dry heat, the requiring a higher temperature and the necessity of a longer period of time for treatment, due to the slow rate of penetration through dry air, seems to change the character of the bread to a less degree than does the autoclave treatment. The results show that the time of molding of bread may be materially postponed by means of treating the wrapped loaf with heat. The growth of mold spores is inhibited and it is possible practically to postpone indefinitely the molding action. This, of course, is neither desirable nor necessary, due to the aging of the product and its limited life. By varying the temperature and time relations it is possible to determine the optimum conditions for the specific case; some bakers may desire the product to keep longer than do others.

In all of these experiments, the type of container used was one made of tin especially designed for the purpose. These containers keep the loaves remarkably fresh; a loaf will keep for from two to three weeks without drying out. Of course, due to the incubating action, optimum conditions are present for mold growth, but if the loaf can be treated so that the growth of the mold is inhibited, it seems that some method might be possible on a commercial scale; a method adapted to use tin containers would keep the loaves in a very satisfactory condition and would prolong the life of the loaf to a marked degree. This is merely of interest in passing as several objections must be overcome before such a process could be applied commercially.

2. CHEMICAL TREATMENT. The search for a chemical which possesses sufficient disinfecting power to destroy mold spores, or inhibit their action, which is not harmful when taken into the body, and which when applied to bread does not change its character, has proved practically in vain. The Pure Food Laws are so carefully drawn and allow for the use of such a limited number of antiseptics for the preservation of foodstuffs, that this method of preventing mold growth in the loaf seems rather out of the question.

Experiments were performed using formaldehyde. The infected loaves (loaves wrapped and ready for shipment) were sprayed with solutions of varying dilutions of formaldehyde to determine whether or not this disinfectant would prevent mold growth.

a. One loaf was sprayed with a solution (diluted 1:50,000).

b. One loaf was sprayed with a solution (diluted 1:25,000).

c. One loaf was sprayed with a solution (diluted 1:250).

These loaves so treated were placed in sterile tin containers and were examined at the end of seven days.

a. Loaf showed growth of Penicillium, Aspergillus, and Rhizopus.

b. Loaf showed growth of Penicillium, Aspergillus and Rhizopus.

c. Loaf showed growth of Penicillium, Aspergillus and Rhizopus.

The types found on these loaves were the same, as was also the degree of infection, irrespective of the dilution.

Formaldehyde forms a compound with the protein present in bread which cannot be detected, if used in a sufficiently high dilution. The formaldehyde cannot be detected in a dilution of one part in fifty thousand. If this dilution was sufficiently potent to destroy the mold spores or inhibit their growth, the situation would be ideal; the formaldehyde could inhibit the action of the mold and then form an unrecognizable compound (unrecognizable as far as formaldehyde is concerned).

Unfortunately, as the above results bear out, the concentration of formaldehyde necessary to inhibit mold growth is much too high to make possible its use in this case.

An attempt was made to treat the bread wrapping paper with formaldehyde, considering that the moisture given off by the bread would be sufficient to bring the formaldehyde into solution and then inhibit mold growth. The paper was coated with a solution of formaldehyde and the infected loaf was wrapped.

Two portions of paper were treated: One sample was treated with a dilution (sprayed) of 1:50,000, and the other was treated with a dilution of 1:250. Both loaves showed growth of Rhizopus and Aspergillus at the end of seven days.

One loaf was sprayed with a solution of formaldehyde 1:50,000, and was wrapped with wrapping paper which had been treated with the same dilution of chemical. The loaf was examined at the end of seven days and showed a growth of Rhizopus and Penicillium.

The chemical treatment of the crust of the loaf or the surface of the paper seems to be out of the question. Even if a chemical could be found or synthesized which would prevent mold development and which was allowed by the Pure Food Laws, it would be necessary that this information appear on the label and this would undoubtedly interfere with the sale of the bread.

3. PHYSICAL TREATMENT. Several experiments were carried out to determine if it might not be possible to treat the loaf of bread with some substance which would form a thin coating over the surface and thus mechanically prevent the molds from developing.

a. Four loaves were coated with a mixture of egg albumin and sugar. The mixture produced a thin, dry, transparent film over the surface of the loaf and added a rather pleasing appearance to the product. (The mixture could be applied either by spray or brush). The loaves so treated were placed in sterile tin containers and examined at the end of seven days. A potent growth of Aspergillus and Rhizopus was found on all of the loaves; the loaves used in this experiment were infected with mold.

b. Several loaves were infected with mold (Penicillium and Aspergillus), were coated with a thin film of paraffin and allowed to remain in this condition for varying periods of time. The molds were found to grow on the surface of the bread despite the film of paraffin. The paraffin serves to retain the moisture of the loaf very efficiently; the loaves examined were not very much dried out at the end of two weeks.

Methods for preventing the growth of mold by mechanical methods have proved fruitless. There remains one other possible method of preventing mold growth and that is by rendering conditions unfavorable for the growth of the molds.

Two loaves, which had been infected with Penicillium, Aspergillus and Rhizopus, were placed in sterile glass containers. One loaf was placed in the ice chest (15.5C), the other was placed in the 37°C. incubator. The moisture conditions remained practically constant, due to the fact that the containers were tightly sealed, effectually retaining the moisture of the original loaf. The growth of mold on these loaves was observed daily.

15.5°C.—no growth of mold was observed up to the eleventh day.

37°C.—5th day, heavy growth of Aspergillus, Rhizopus and Penicillium.

Low temperatures inhibit mold growth; body temperatures favor growth.

Four loaves were placed in the incubator (not in tin containers). These loaves were examined up to fourteen days. No growth of mold took place. The loaves had lost a large amount of moisture and the crust was hard, thus offering very unfavorable conditions for mold growth. The moisture of the surroundings is thus more important than the moisture of the bread. Dry air discourages mold growth, as does also air in motion. Growth is proportional to the moisture of the air and the bread. Dry bread in moist places will mold more quickly than moist bread in dry places.

Experiments were carried out exposing molds to reduced amounts of oxygen. The molds do not grow as well under anaerobic conditions but they do not grow—thus the oxygen content is not important.

Two loaves were infected at the center with a culture of Penicillium mold and were then coated with a film of paraffin. The loaves showed a very heavy growth of mold at the end of seven days. These loaves were inoculated after they had cooled, therefore, sufficient time had elapsed for the exchange of gases, the oxygen content of the loaf had increased and the carbon dioxide decreased. Though the oxygen content of the loaf did not represent atmospheric conditions, the amount of oxygen was, no doubt, considerable. The point brought out by this experiment is that the prevention of mold growth by decreasing the oxygen supply seems impractical, unless the amount of oxygen is decreased to a very low level. The experiments performed, maintaining cultures of various molds, under anaerobic conditions, tend to bear out this point. It is of no avail to maintain anaerobic conditions for the loaf, as sufficient oxygen to support the mold growth is present in the center of the loaf.

The temperature and moisture relations are important factors in preventing mold growth. Heat treatment of the wrapped loaf promises to be of value. Chemical treatment and mechanical coating of the bread did not bear fruitful results, hence these methods are deemed unsatisfactory for practical application.

A. The second method of preventing mold growth on bread is that of excluding the mold spores from the loaf. Mold spores are ubiquitous wherever the temperature is not so high as to destroy life. These spores are present, as previous experiments have shown, in the air, on the machines, on the walls, etc., of the bakery, in fact, they are everywhere. The most common medium of dispersion is probably the air.

The baked loaf of bread, which is sterile, after leaving the oven is placed on bread cooling racks for about thirty minutes before it is wrapped. The mold spores are ever present to infect the bread during this process, and as stated above, practically every utensil with which the bread comes in contact, is infected. It is necessary, if infection of the bread with mold spores is to be prevented, that some method be devised to protect the bread so that these spores cannot gain access.

One method which might be installed is to make use of a separate room for cooling the bread, which is to go long distances. The air which enters this room should be filtered and rendered sterile, the bread racks should be treated with live steam to destroy mold spores present and the workers should be supplied with sterile uniforms. Of course the room should be disinfected previous to the operation of this method. The baked loaves could be immediately transferred to this room, after leaving the oven, and cooled under aseptic conditions. The wrapping machines could be installed in a similar room. The machines could be rendered free from mold spores by treating them with live steam. Since the paper is infected mainly from the dust-laden air of the bakery, by using these precautions the paper would be practically free from infection. By using these methods of maintaining aseptic conditions of handling the bread during the cooling and wrapping processes, the source of infection is eliminated. It is, of course, unnecessary to maintain such conditions throughout the bakery; it is merely necessary to separate the cooling and wrapping processes from the general process.

The two methods of preventing the molding of bread which seem to be the most practical and efficient are the heat treatment of the wrapped loaf, not using any precautions to keep the bread from contact with infection during the cooling, and wrapping the loaf maintaining aseptic conditions. Which method of treatment to be the more efficient will naturally depend largely on the expense necessary to carry out the process. No estimates have been made comparing the approximate cost of the two methods. The problem is for the engineer to solve.

STUDY OF MOLD TYPES.

At the beginning of this work considerable time was devoted to an endeavor to classify the molds which were found to be present on bread. This was rather superficial at best, and on conferring with authorities and giving consideration to the problem at hand, it was decided that physiological characteristics rather than morphological were to be desired. Consequently the field was limited to the main types and some of the important physiological phases are herewith presented.

Penicillium, Aspergillus, and Rhizopus were found to be constantly present in nearly all cases wherein the molding of bread is involved. No necessity for an identification into species was brought to mind since there is little distinction within the genus, and the characteristics are to a large extent overlapping.

ANAEROBIC CULTURES. Using dextrose agar slants as a medium, Aspergillus, Penicillium and Rhizopus were submitted to anaerobic conditions, using the Sodium Hydroxide and Pyrogallic acid method. They were allowed to incubate at 20° C.

On the second day after anaerobic conditions had been set up, mold growth was exhibited by all tubes. The growth was not strong, abundant or characteristic and fruiting structures appeared with little regularity or not at all. However, the spores do manage to send out mycelia to a small extent without abundant oxygen being present.

The general characteristics of the three main types may be correlated as follows:

Rhizopus nigricans (Mucor stolonifer)
- Occurrence—exterior of loaf when moist, and in flour.
- Temperatures—10° C. to 30° C.
- Appearance—At first white, becoming gray to black. Mycelia colorless. Is not rapid in growth.

Aspergillus glaucus
- Occurrence—exterior of loaf and in flour
- Temperatures—10° C. and 30° C.
- Appearance—At first white, then blue-green to gray-green with a white halo, later grayish, in old cultures gold, due to the formation of perithica. Mycelia colorless to yellow when perithica are present

Penicillium crustaceum
- Occurrence—exterior of loaf and in flour
- Temperatures—10° to 25° C.
- Appearance—White to blue-green later gray blue-green or dark gray. Mycelia are colorless. Is least rapid in growth.

The above characteristics are quoted merely as general information and much work that has been done by workers on this phase of the subject is purely scientific. Any standard work on Cryptogamic

Botany or on Fungi contains ample data on this subject.

As for the temperatures, above 55° C. no molds exist, the lower limit being about 10° C.

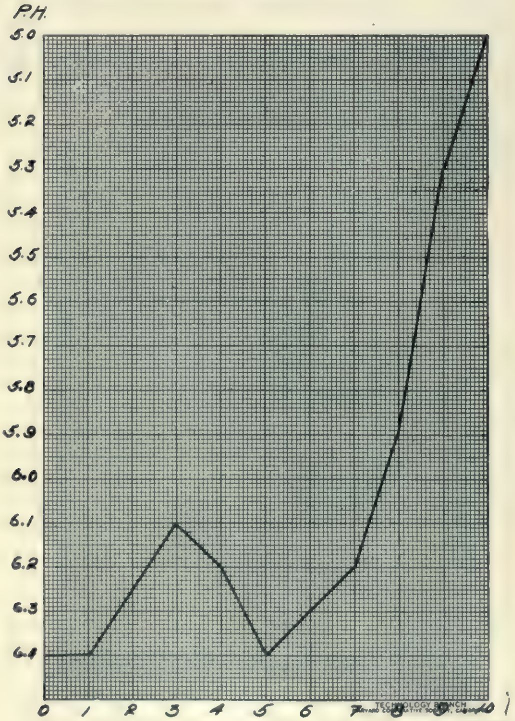
BACTERIA.

On several occasions bread which had been kept sterile for two to three weeks exhibited a sliminess on the edges which was at first thought to be due to the condensed moisture in the container. However, on taking cultures from this material, an organism was found which, when obtained in pure culture, exhibited the following characteristics:

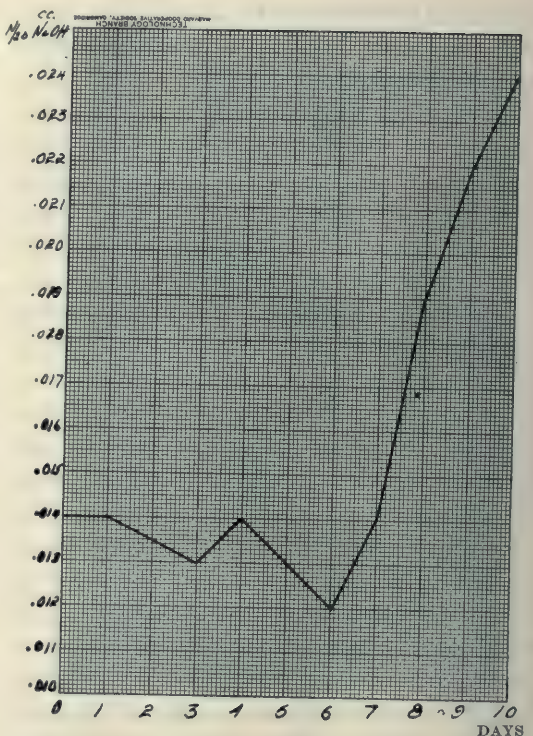
Morphology—Bacilli .8 x 1.6—5.0. Stain by Gram's method.

Gelatin colonies—without radiations. Surface colonies; round, becoming crateriform, with a grayish white, delicate, crumpled membrane.

Gelatin stab—in depth, a narrow tube; on the surface, a crateriform to stratiform liquefaction, with surfmembrane.



Showing P.H. Reactions as Described



Showing changes in acidity titratable with N/20 NaOH.

Agar slant—Growth gray white, glistening, becoming crumpled.

Bouillon—weakly turbid; membrane which is firm and not easily broken by shaking.

Milk—coagulating slowly, alkaline, peptonized.

Potato—Growth thin, white, crumpled, becoming light yellowish.

Indol—negative.

Identified as B. mesentericus vulgatus.

DETERMINATION OF ACIDITIES.

In order to ascertain whether or not the acidity of the crust of the loaf changed so as to either favor or inhibit mold growth, experiments were carried out to determine the titratable and actual acidities. Two loaves were collected which were wrapped and were ready for shipment. Determinations of the hydrogen-ion concentration and titratable acidity were made daily, covering a period of ten days, from samples of these two loaves, one loaf serving as a check on the other. The samples used in making these determinations averaged from one to two grams in weight and were collected from the crust of the loaf with not more than one-half inch of the crumb included. The samples were titrated and taken up with 200 cc. of distilled water (PH=6.4). The hydrogen-ion concentration was determined, using the colormetric method. The acidity (titratable) was determined by titrating 200 cc. of the sample with N/20 sodium hydroxide; brom thymol blue and methyl red were used as indicators. The results obtained are as follows:

	PH		Acidity titratable N/20 NaOH
1st day	6.4		.014
3rd day	6.1		.013
4th day	6.2		.014
5th day	6.4		.013
6th day	6.3		.012
7th day	6.2		.014
8th day	5.9	heavy growth of mold	.019
9th day	5.3		.022
10th day	5.0		.024

These results show that the acidity of the loaf is practically constant during the first seven days of its life. The loaves showed an abundant growth of mold at the end of eight days and the acidity (titratable) underwent a marked increase when the PH decreased considerably, as was to be expected. Whether the mold produced a rise in acidity due to its action on the substrate or the bread, due to bacterial action, increased in acidity, is not known; most likely the mold was the cause of the action and not the result. Consequently, it may be said that there is a relation between the degree of acidity and the growth of mold. (The PH varies slightly for the first seven days, but it is to be noted that a change of PH of .1 is very small, almost negligible in this case.)

There were two phases of this work which, due to lack of time, were not investigated. The relation of colloid chemistry to the molding of bread seems rather remote, but a brief consideration of a phenomena of colloids, in regard to bread, serves to show a possible relationship. During the process of aging, bread undergoes a drying out process, known in colloid chemistry as ''Syneresis.'' As a result of this process there forms over the surface of the loaf an invisible film due to the influence of the loss of water on the colloidal system. This film contains a nutrient material and whether or not this serves as a favorable medium for the development of mold is not known; it seems, however, that this may be the case. This film may be driven back into the loaf and thus assume its original state by a process of secondary heating; this serves also to prolong the life of the loaf.

The other consideration which may have an application to this problem is the disinfecting power of hydrogen-ions. It is known that the effect of hydrogen-ions on the accumulation and activation of enzymes produced by certain fungi is marked, between certain limits; it may be possible to so regulate the hydrogen-ion concentration that the development of mold can be prevented. There has been some work done upon these problems by several investigators, but not with regard to the problem of bread molding. It seems that this problem offers a fruitful field for investigation.

GENERAL SUMMARY OF CONCLUSIONS:

1. The amount of mold infection is proportional to the sanitary condition of the bakery and decreases as this condition approaches excellence.

2. The baking process is one of sterilization as far as molds are concerned, the loaf being sterile when it leaves the oven.

3. Preheating of ingredients is not necessary.

4. The maximum temperature at the center of the loaf is 97° C.

5. Mold infection takes place after the bread leaves the oven.

6. Mold growth starts on the exterior and ingresses; a heavy crust being unfavorable to such a growth.

7. Infection is due to air, handling, racks, machines, and paper.

8. The types of molds found consistently on moldy bread are Aspergillus, Penicillium and Rhizopus. Sporadically, Mucor and fungi imperfecti have been observed.

9. Moisture in the air and in the substratum are necessary for mold growth, the former being the more important.

10. Clean, cool air and sunlight have an inhibitory action on the growth of molds.

11. Mold infection may be prevented to a large extent by sanitary precautions, such as (1) daily scrubbing of floors, (2) frequent washing of walls and ceilings with antiseptic solutions, (3) filtering and washing of air in cooling and wrapping rooms, (4) use of live steam on machinery and conveyors, racks, etc.

12. The development of mold growth may be postponed by heat treatment of the wrapped loaf; the time of postponement being determined by the time and intensity of the heat treatment.

13. The application to the loaf of mold preventives has met with little success.

14. Wrapping paper is in general infected with mold spores, infection usually being accomplished in the baking.

15. Certain bacteria may survive baking.

16. The mycelia of molds are capable of penetrating the wrapping paper when moist.

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